

This listing of claims presented below replaces all prior versions and listings of claims in the application.

Listing of Claims

Claims 1-36 (cancel)

37. (Previously Presented) An isolated enzyme product of plant origin designated NPPase, characterized in that it catalyses the hydrolysis of nucleotide sugars in equimolar conditions to sugar-phosphate and corresponding nucleoside monophosphate, is able to hydrolyse bis-PNPP, is stable at pH between 4 and 7.5, and has an apparent molecular weight determined by gel filtration around 70 kDa for the monomeric form and around 270 kDa for the homopolymeric form.

38. (Previously Presented) The enzyme product according to claim 37, characterized in that: it does not hydrolyse, GlP, G6P, AMP, 3-phosphoglycerate, AMPc and nucleic acids; it is inhibited by orthophosphate, inorganic pyrophosphate, and phosphate esters; it is resistant to Proteinase K or Pronase; and it recognizes as substrates, ADPG, UDPG, GDP-glucose, ADP-mannose, APS, PAPS and bis-PNPP.

39. (Previously Presented) The enzyme product according to claim 37, that is resistant at a temperature of 65°C for 30 minutes, displays a K_{eq} of reaction of 110 and its $\Delta G'$ is -2.9 kcal/mol.

40. (Previously Presented) The enzyme product according to claim 37 that is isolated from barley.

41. (Previously Presented) The enzyme product according to claim 37 wherein the amino

acid sequence of its N-terminal end is as represented by SEQ ID NO:1, and its amino acid sequence contains internal sequences represented by SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

42. (Previously Presented) The enzyme product according to claim 40 wherein the amino acid sequence of its N-terminal end is as represented by SEQ ID NO:1, and its amino acid sequence contains internal sequences represented by SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

43. (Previously Presented) The enzyme product according to claim 41, that contains a sequence represented by SEQ ID NO:23.

44. (Previously Presented) The enzyme product according to claim 38, wherein its K_m for ADPG 15 is 0.5 mM.

45. (Previously Presented) The enzyme product according to claim 37 that is isolated from rice.

46. (Previously Presented) The enzyme product according to claim 37 wherein the amino acid sequence of its N-terminal end is as represented by SEQ ID NO:7, and its amino acid sequence contains internal sequences represented by SEQ ID NO:8, SEQ ID NO:9, 20 SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO:16 and SEQ ID NO:17.

47. (Previously Presented) The enzyme product according to claim 45 wherein the amino acid sequence of its N-terminal end is as represented by SEQ ID NO:7, and its amino acid sequence contains internal sequences represented by SEQ ID NO:8, SEQ ID NO:9, 20 SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO:16 and SEQ ID NO:17.

48. (Previously Presented) The enzyme product according to claim 46 that includes a sequence represented by SEQ ID NO:21.

49. (Previously Presented) The enzyme product according to claim 46 wherein its K_m for ADPG is 0.60 mM.

50. (Previously Presented) A method for isolating an enzyme product of plant origin according to claim 37 comprising the steps of:

- a) extracting a protein fraction from plant material using a buffer to obtain an extract;
- b) filtering the extract and purifying the extract by centrifugation and precipitation with adjustments both of pH and ionic strength of medium, and
- c) purifying the extract by gel filtration, isoelectric focusing, denaturing-gel electrophoresis, or other equivalent means of purification of proteins extracted from the plant.

51. (Previously Presented) The method according to claim 50 wherein in step b) the extract is heated to above 60°C and then cooled in ice.

52. (Previously Presented) The method according to claim 50, wherein in step a) the plant material is plant tissue and the plant tissue is homogenized with an extraction buffer, type Mes 50 mM pH 6, EDTA 1 mM, DTT 2 mM; in step b) the extract is ultracentrifuged at 100 000 g and precipitated using ammonium sulphate and the precipitate is resuspended in a buffer of pH 4.2 to form a solution, the solution is heated for at least 15 minutes at a temperature between 60 and 65°C, then cooled in ice, centrifuging at 30 000 g to obtain a supernatant and protein in the supernatant, concentrating the protein of the supernatant by precipitation in ammonium sulphate, resuspending the protein in a buffer of pH 6, and in step c) purifying the protein by gel-filtration chromatography, isoelectric focusing or denaturing-gel electrophoresis.

Claims 53-74 (cancel)

75. (New) Method for producing a transgenic plant having a reduced amount of starch, cell wall polysaccharides or both, said plant is also resistant to high temperatures and salinity, as compared with a wild type plant grown under the same conditions, comprising transforming a wild type plant with an expression vector comprising a cDNA sequence which is expressed or over expressed in the transformed plant and encodes for a enzyme having NPPase activity.

76. (New) Method according to claim 75, wherein the cDNA sequence is selected from SEQ ID NO: 20 and SEQ ID NO: 22.

77. (New) Method according to claim 75, wherein the cDNA sequence is obtained from a cDNA library by using a cDNA amplified with a 5' primer SEQ ID NO: 18 and with a 3' primer SEQ ID NO: 19 as probes.

78. (New) Method according to claim 75, wherein the wild type plant is transformed with *Agrobacterium tumefaciens* comprising the cDNA encoding for NPPase enzyme activity.

79. (New) Method according to claim 78, wherein the wild type plant is transformed with *Agrobacterium tumefaciens* CECT 5799.

80. (New) Method according to claim 75, wherein the transgenic plant is a tobacco, potato or tomato plant.

81. (New) Primer of SEQ ID NO: 18.

82. (New) Primer of SEQ ID NO: 19.

83. (New) cDNA represented by SEQ ID NO: 20 encoding for NPPase activity.

84. (New) cDNA represented by SEQ ID NO: 20 encoding for an NPPase enzyme activity.

85. (New) Transgenic plant transformed with an expression vector comprising cDNA

sequence encoding an enzyme having NPPase activity wherein the plant has a reduced amount of starch, cell wall polysaccharides or both and, is also resistant to high temperatures and salinity, as compared with a wild type plant grown under the same conditions.

86. (New) Transgenic plant, according to claim 85, wherein the cDNA sequence is selected from SEQ ID NO: 20 and SEQ ID NO: 22.